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BIODIVERSITY RESEARCH

Phylogeographic consequences of different introduction histories of invasive Australian *Acacia* species and *Paraserianthes lophantha* (Fabaceae) in South Africa

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ABSTRACT

Aim The genetic makeup and evolutionary potential of alien species can be profoundly influenced by their introduction history, but without detailed historical records, it can be difficult to ascertain the strength of this historical contingency. We explore how the known introduction histories combined with phylogeographic patterns in the native range have affected the genetic diversity in the invasive range for five Australian trees introduced to South Africa (*Acacia cyclops*, *Acacia mearnsii*, *Acacia pycnantha*, *Acacia saligna* and *Paraserianthes lophantha*).

Location Western Australia, South Australia, Victoria and New South Wales (native and invasive ranges), and South Africa and the Hawaiian Islands (invasive range).

Methods DNA sequence data were generated for all study species for either the external transcribed spacer (ETS) or plastid rpl32-trnL$^{(UAG)}$ gene regions (combined total of 180 DNA sequences). Using statistical parsimony networks and genetic diversity indices, we compared genetic structure and variation in native and invasive ranges.

Results Australian acacia species tend to have high genetic diversity at the population level in their native ranges, often showing high intra-specific divergence. In most instances, these species have similar levels of population genetic diversity in their adventive ranges in South Africa, but lack structure. For *A. cyclops*, *A. saligna* and *P. lophantha*, we found evidence for intra-specific hybridization between mixed genetic entities in the invasive range, arguably as a result of the structured native range being broadly sampled prior to introduction.

Main conclusions Invasive species that have been extensively used in forestry often have complex introduction histories resulting in equally complex genetic signatures in the invasive range. Our results show that extreme caution should be taken when using indirect inferences (molecular genetic data) of introduction histories in the absence of detailed introduction records.

Keywords
***Acacia*, biological invasions, genetic diversity, introduction history, invasive species, phylogeography.**

INTRODUCTION

Determining the attributes that make some plant species more successful than others at colonizing and invading new environments remains difficult. However, introduction effort and its effects on propagule pressure have been shown to be crucial for successful plant invasions (Simberloff, 2009). Intuitively, introduction effort has the potential to influence

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genetic diversity, by cointroducing previously allopatric genetic entities, or introducing pre-adapted genotypes. It is therefore not surprising that multiple introductions are often associated with successful invasions (Bossdorf *et al.*, 2005). Multiple introductions can help overcome the negative effects of founder events and genetic bottlenecks so that newly established populations can, and often do, harbour similar levels of genetic diversity to that in native populations (Lavergne & Molofsky, 2007). Moreover, multiple introductions from isolated native populations could lead to new genetic combinations and genotypic novelty in the invasive range (Prentis *et al.*, 2008; Rosenthal *et al.*, 2008). However, the level and pattern of genetic diversity in the native range will also have a significant influence on the level of genetic diversity introduced. For example, signatures of high genetic diversity in an invasive species may have arisen through multiple introductions from highly diverse sources or from a single introduction of a source derived from multiple populations (Fig. 1). In such instances, without access to detailed introduction histories, it can be difficult to be confident about distinguishing genetic signatures resulting from single introductions from those of multiple introductions (Eales *et al.*, 2010; Fig. 1). Therefore, while some genetic signatures may be definitive in relation to introduction history and native range genetic diversity (Wilson *et al.*, 2009), many will be inconclusive without additional information.

Most investigations of the genetic consequences of introduction histories have relied on comparisons between genetic diversities in native and invasive ranges to infer introduction effort, source and pathway (Estoup & Guillemaud, 2010). Very few studies have addressed the influence of known introduction history on the standing genetic diversity in contemporary populations of invasive species (but see Eales *et al.*, 2010). This is partly because of the difficulty of obtaining estimates of genetic diversity in both introduced and native ranges as well as accurate accounts of introduction history. Invasive plant species that have been extensively used in forestry applications may not be constrained by these limitations as their introduction histories are often well documented (Richardson, 1998). Moreover, in some instances, multiple species within a single genus have been introduced globally for silvicultural use (e.g. *Acacia* Mill., *Eucalyptus* L'Hér. and *Pinus* L.), making multiple-species comparisons within this framework possible (Richardson *et al.*, 2011). Comparison of introduction histories and genetic diversity between native and invasive ranges in different species within the same genus has the potential for yielding important biogeographical insights into the interaction between introduction scenarios and native genetic diversity and structure. Many woody legumes have been extensively planted outside their natural ranges all over the world for many purposes, and many taxa are now naturalized or invasive. Richardson & Rejmánek (2011) list 123 woody legumes that qualify as 'invasive' following the criteria of Pyšek *et al.* (2004). Currently, 23 Australian taxa of *Acacia* subgenus *Phyllodinae*, commonly known as wattles, are considered invasive, i.e. spreading naturally from introduction sites in regions far removed from their native range (Richardson & Rejmánek, 2011). This group features prominently in the invasive flora of South Africa. Ten of the 100 most widespread

Introduction scenario	Genetic signature expected in invasive range(s)	Alternative interpretation without detailed introduction records	Example from current study
(1) Single introduction from multiple combined sources (potentially also including other invasive ranges) to a single location.	Genetic diversity representative of native range (possibly admixture if genetic structure present in native range). Diversity highest at introduction site.	Multiple introductions from distinct native sources.	*Acacia cyclops* 
(2) Multiple introductions from different sources to multiple locations.	Genetic diversity representative of native range (possibly admixture). Genetic diversity likely structured.	None - multiple introductions from distinct sources	*Acacia mearnsii* 
(3) Multiple introductions from the a geographically restricted source to a geographically restricted location.	Genetic diversity low (possible bottleneck) compared to overall native range diversity.	Single introduction from very low (possibly single) native source.	*Acacia saligna* 
(4) Multiple introductions from different sources to one location.	Genetic diversity representative of native range (possibly admixture). Genetic diversity likely **not** structured.	None - but potentially multiple introductions to multiple locations.	*Acacia pycnantha* 
(5) Multiple introductions from the same source to one location.	Genetic diversity low (possible bottleneck) compared to overall native range diversity.	Single introduction from very low (possibly single) native source.	Not applicable
(6) Single introduction from a single source population to a single location.	Genetic diversity extremely low and bottlenecked compared to overall native range diversity.	None - single introduction event	Not applicable

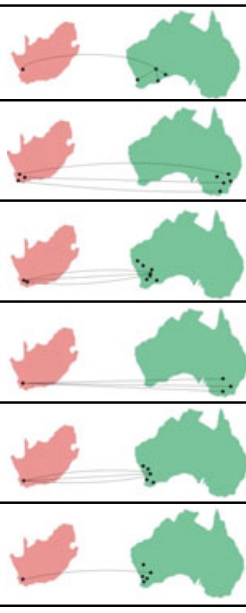

Figure 1 Different introductions scenarios are predicted to leave distinct genetic signatures in the invasive range(s) of introduced species. However, without detailed introduction records, indirect genetic estimates of the number(s) and source(s) of introductions may lead to erroneous conclusions. The figure summarizes some of these aspects for introductions from *genetically structured* native range populations and assumes no environmental filtering (selection) in the invaded regions (which may dramatically alter patterns).

invasive plants in South Africa are wattles, and *Acacia mearnsii* De Wild. is the fifth most widespread invasive plant species in South Africa (Fig. 2) (Henderson, 2001). While some acacias are very widespread in South Africa, others are much less widely distributed, e.g. *Acacia pycnantha* Benth. (Fig. 2), and others still are limited to only one or two sites (Zenni *et al.*, 2009; Wilson *et al.*, 2011).

Australian wattles were introduced to South Africa for three main reasons: (1) tannin production and associated forestry purposes (i.e. the so-called tanbark wattles, *Acacia dealbata*

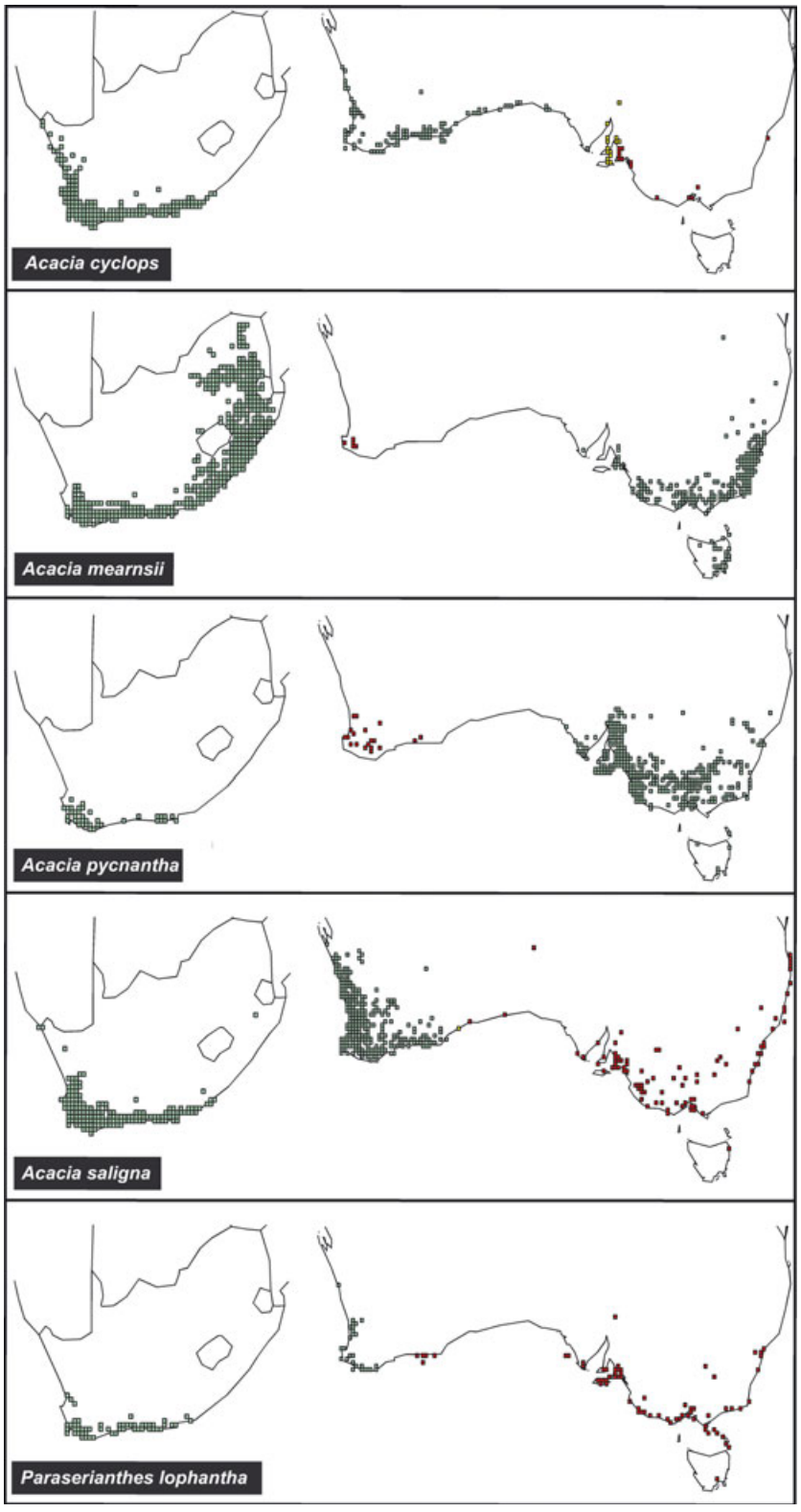

Figure 2 Distribution ranges (at quarter-degree resolution) of species from this study in their native ranges in Australia (right) and their invasive ranges in South Africa (left). Australian records outside the natural range of the species are indicated in red; those with questionable native/naturalized status are indicated in yellow.

Link, *A. decurrens* Willd. and *A. mearnsii*); (2) stabilization of coastal dunes (*Acacia cyclops* A. Cunn. ex G. Don, *Acacia saligna* (Labill.) H.L. Wendl.; (3) ornamental purposes (e.g. *A. elata* A. Cunn. ex Benth.).

The history of wattle introductions to South Africa has been well documented (Shaughnessy, 1980; Poynton, 2009), and for some species, the scale of these introductions is impressive. For example, between 1850 and 1985, *A. mearnsii* was introduced on at least eight independent occasions – in 1893, an estimated 1.1–2.5 million seeds were introduced in a single consignment (Poynton, 2009). In comparison, *A. pycnantha* was introduced from Australia only twice, although there was extensive transfer of seed within South Africa (Poynton, 2009).

The detailed accounts of introductions to South Africa make Australian acacias an ideal system for studying the effects of introduction history on genetic diversity, population genetic structure, adaptive potential, genome-wide processes and potentially invasiveness. Acacias also generally have high genetic diversity in their native ranges (Broadhurst *et al.*, 2001; Byrne *et al.*, 2001, 2002; Coates *et al.*, 2006; George *et al.*, 2006; Millar *et al.*, 2008) making inferences about source of introductions more definitive. In this paper, we selected five species for which to contrast different introduction histories with phylogeographic structures in the native and invasive ranges. In particular, we address the following questions: (1) How do introduction histories affect genetic diversity within invasive ranges? (2) Are there common phylogeographic patterns in Australia such that multiple introductions from the native range coincide with admixture of genetically distinct and allopatric entities in the invasive range? (3) What are the potential implications of diverse introductions for management of invasive acacias in South Africa?

METHODS

Study species

We considered five species – *A. cyclops* A. Cunn. ex G. Don, *A. mearnsii* De Wild., *A. pycnantha* Benth., *A. saligna* (Labill.) H.L. Wendl. and *Paraserianthes lophantha* (Willd.) I.C. Nielsen (a closely related species of *Acacia* subgenus *Phyllodineae*; Brown *et al.*, 2008) – that represent a range of invasions, from species that were introduced multiple times, planted commercially, and that are now widespread invaders (e.g. *A. mearnsii*), to species that were introduced on only a few occasions, were never widely cultivated and have sparse invasive ranges (e.g. *P. lophantha*). Details of introduction histories into South Africa for all study species are given in Table 1.

Sampling and DNA extraction

Leaf material was collected from across the native (i.e. Australia) and invasive (South Africa) ranges for each species from at least six localities (range 6–12) per country. Where available, additional invasive populations from Australia were also included, and for *P. lophantha*, material from the Hawaiian Islands was included [again the invasive range but supposedly a different subspecies (i.e. subsp. *montana*) to that native in Australia and invasive in South Africa (i.e. subsp. *lophantha*)]. Leaf material was dried and kept on silica gel until further use. Herbarium specimens were lodged for all populations [State Herbarium of South Australia (AD); Western Australian Herbarium (PERTH); or University of Stellenbosch Herbarium (STEU)].

Total genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) extraction protocol described by Doyle & Doyle (1990) with the addition of 0.2 M sodium sulphite to the extraction and wash buffers.

PCR amplification and DNA sequencing

One nuclear region, the external transcribed spacer region (ETS), was amplified using primers described in Brown *et al.* (2008) in all species except *A. pycnantha*. For *A. pycnantha*, we amplified the rpl32-trnL$^{(UAG)}$ chloroplast intergenic spacer region using primers described by Shaw *et al.* (2007). For both gene regions, polymerase chain reactions (PCR) were performed in 50-μL volumes, each containing approximately 50 ng of genomic DNA, 200 μM of each dNTP (AB gene; Southern Cross Biotechnologies, Cape Town, South Africa), 25 pmol of each primer, 5 U *Taq* DNA polymerase (Super-Therm JMR-801; Southern Cross Biotechnologies), 1× PCR reaction buffer, 1.5 mM $MgCl_2$. PCR cycling was performed at initial denaturation of 95 °C for 5 min, followed by 35 cycles at denaturation at 94 °C for 30 s, annealing at 62 °C for 60 s, elongation at 72 °C for 90 s; and final extension at 72 °C for 10 min. Amplified DNA fragments were purified using the QIAquick PCR Purification kit (Qiagen, Cape Town, South Africa, Southern Cross Biotechnologies) and sequenced in one direction (with forward primer) using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and an automated ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). The short lengths of both genes meant that reads were unambiguous even when sequenced in only one direction. In almost all cases, direct sequencing of PCR products produced clean and unambiguous nuclear gene sequences. However, in a few instances, we identified one or more ambiguous base pairs. For these accessions, we cloned ETS PCR products using the pGEM®-T Easy Vector System (Promega, Whitehead Scientific, Cape Town, South Africa) and sequenced a number of positive inserts until both copies were retrieved.

DNA sequence alignment and analysis

Contiguous sequences were constructed, edited and aligned in BioEdit version 7.0.5.3 (Hall, 1999). All edited sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov, Table S1 in Supporting Information). We also included data for those *Acacia* and *Paraserianthes* taxa that had ETS data available on GenBank.

Sequence data were used to reconstruct phylogenetic networks using statistical parsimony (Templeton *et al.*, 1992)

Table 1 Summary of the introduction histories for Australia *Acacia* species and *Paraserianthes lophantha* to South Africa (adapted from Poynton, 2009 and Stirton, 1978).

Species	Date(s)	Source	Introduction effort (# seeds)	Notes
Acacia cyclops	1845	Australia	Unknown	Presumably a large seed stock
	1895	France	2600–5600	
Acacia mearnsii	1850	Australia	Unknown	
	1858	Australia	Unknown	
	1860	Australia	Unknown	Two 'packets' of seed
	1864	Australia	Unknown	Most likely small quantity of seeds
	1893	Australia	1.1–2.5 million	Possibly more than a single introduction
	1931	Australia	2400–5500	
	1957	Australia	Unknown	Seeds collected from 25 localities in NSW, VIC, SA and TAS
	1985	Australia	Unknown	Seeds collected from 9 localities in NSW and VIC
Acacia pycnantha	1865	Australia	Unknown	
	1893	Australia	Unknown	
	1893–1910	South Africa	22.5–29.1 million	Locally sourced from plantations
Acacia saligna	1845	Australia	Unknown	
	1884–1892	Unknown	224–281 million	Source unknown but potentially sourced in South Africa
	1896	France	55,000–65,000	Approximate introduction effort since between 110,000–137,500 seeds were imported between 1896–1901
	1901	France	55,000–65,000	See note above
	1922	Australia	Unknown	Most likely small quantity of seeds
Paraserianthes lophantha	1833	Australia	Unknown	Most likely small quantity of seeds
	1835	Australia	Unknown	

NSW, New South Wales; VIC, Victoria; SA, South Australia; TAS, Tasmania.

as implemented in the TCS 1.13 software (Clement *et al.*, 2000). We chose network over traditional phylogenetic tree building approaches to visualize and explore the data, not only because this approach renders higher resolution, but also because networks are better suited for exploring relationships between genes sampled within a species, as these are often not hierarchical as assumed by traditional phylogenetic tree reconstruction methods (Posada & Crandall, 2001).

Levels of genetic diversity among native and invasive regions were computed as the mean number of pair wise sequence differences within each geographic region (Australia and South Africa). We also computed nucleotide diversity, an estimate of the probability that two randomly selected homologous loci (nucleotides) are different (Tajima, 1983; Nei, 1987). All computations were carried out in ARLEQUIN version 3.5 (Excoffier & Lischer, 2010).

RESULTS

DNA sequence variation

Aligned ETS sequence data ranged from 411 bp (*A. cyclops*) to 453 bp (*A. mearnsii*) across all species and aligned rpl32-trnL$^{(UAG)}$ sequences were 602 bp long (*A. pycnantha*). For ETS, alignments gaps ranged from one (*A. cyclops* and *P. lophantha*) to 69 (*A. saligna*). For *A. pycnantha*, gap sizes in the rpl32-trnL$^{(UAG)}$ alignment ranged between 1 and 5. Table 2 summarizes various sequence diversity indices for each species/country. Some species (*A. cyclops*, *A. pycnantha*, *P. lophantha*) showed similar or higher levels of nucleotide and genetic diversity in South Africa and Australia, whereas others, e.g. *A. saligna* and *A. mearnsii*, are evidently more diverse in their native Australian than in their invasive South African ranges (Table 2).

Phylogenetic diversity and structure

Acacia cyclops

Of the species included here, *A. cyclops* showed a surprising amount of nucleotide variation for the ETS region, and very deep intra-specific phylogenetic divergences. A total of 15 sequence variants were identified out of 35 individuals that fell into two main groups (Fig. 3a). Both groups comprised accessions from both native and invasive ranges. Cloned DNA sequences for South African accessions revealed that these

Table 2 Comparative genetic diversity of *Acacia* species and *Paraserianthes lophantha* from Australia and South Africa.

	Native range				Invasive range			
Species	*n*	H*	π†	MPD‡	*n*	H	π	MPD
Acacia cyclops	26	9	0.0328	13.15	10	9	0.0409	16.9
Acacia mearnsii	16	9	0.006	2.13	7	2	0.0005	0.20
Acacia pycnantha	15	5	0.004	2.54	11	5	0.003	1.73
Acacia saligna	16	12	0.1336	48.09	15	6	0.003	0.93
P. lophantha	19	7	0.0012	0.39	23	6	0.0013	0.51

*Number of haplotypes.
†Nucleotide diversity.
‡Mean number of pairwise differences between all haplotypes.

represented heterozygous individuals, consisting of allelic combinations (hybrids) from both major genetic groups identified. In the native range, all but two populations contained only a single genetic entity. Even those few populations with more than one genotype showed no signs of admixture. Most individual South African populations had multiple alleles representing both major genetic groups (Fig. 3a), in many cases as heterozygote combinations of alleles in potential hybrids.

Acacia mearnsii

Ten haplotypes were identified out of the 25 individuals analysed (Fig. 3b). Invasive populations in South Africa and Western Australia predominantly consisted of a single genetic variant (DNA sequence A) while native populations from south-eastern Australia harboured the remaining genetic diversity (nine unique genetic entities). A single individual (GDT1141, Canberra) was heterozygous (containing alleles A and D).

Acacia pycnantha

Chloroplast rpl32 sequence data identified two major haplotype groups. The majority (eight haplotypes in group one) corresponded to the so-called 'wetland form' of *A. pycnantha* with the remaining haplotype (haplotype I, Fig. 3c) corresponding to the 'dryland form' (M. O'Leary, pers. comm.). Invasive South African accessions were confined to the wetland form (haplotype group one). Haplotypes in the native range appeared structured, with the wetland form (haplotype group one, Fig. 3c) occurring throughout the undulating hills of the coastal belt of South Australia, and large parts of Victoria and New South Wales. The dryland form was restricted to the Flinders Range in South Australia. Within South Africa, the highest haplotype diversity was found in the western parts of the country (initial area of introduction) and only a single haplotype in the eastern parts of the country (secondary introductions arising from South African sources).

Acacia saligna

As with *A. cyclops*, native *A. saligna* ETS sequence data revealed a high amount of nucleotide variation and deep intra-specific phylogeographic divergence with two main groups. A total of 17 ETS sequences were retrieved from 30 individuals falling within the two main groups (Fig 3d). Unlike *A. cyclops*, only a fraction of native range diversity was identified in South Africa. Group I comprised ETS sequences from both native and invasive ranges while those in the second, divergent group were restricted to native range populations (Fig. 3d). Single-copy cloned DNA sequences for South African accession GDT542 (Jeffrey's Bay) revealed allelic combinations (hybrid) between ETS alleles B and D. Admixture was also identified in two Australian populations; accession GDT650 (Wanneroo) and GDT592 and 593 (Tuart Forest), each representing hybrids confined to one of the main ETS clades (Fig. 3d). No allelelic combinations (hybrids) were identified between individuals from the two divergent ETS groups.

Paraserianthes lophantha

Overall, we identified low intra-specific divergence within *P. lophantha*. In its native range, *P. lophantha* had the lowest nucleotide diversity and mean pair wise difference between all haplotypes of all taxa sampled here. A total of nine ETS sequences were identified for the 41 individuals analysed. Native populations from Western Australia harboured six, mostly high frequency, genotypes. Among these, South African populations only shared sequences B and H (Fig. 3e). Interestingly, these high-frequency genotypes were also identified in invasive regions in south-eastern Australia (Fig. 3e). The other haplotype found in the invasive eastern Australian populations (D) was found in the native range, while an additional haplotype found in South Africa (A) was not found in either the native or invasive range in Australia. Hawaiian accessions of *P. lophantha* (GDT198 and GDT773, sequence A) shared ETS sequences with South Africa and differed from Australian accessions by a single mutational step.

DISCUSSION

Multiple introductions are often associated with successful plant invasions partly because of the expected positive effect on

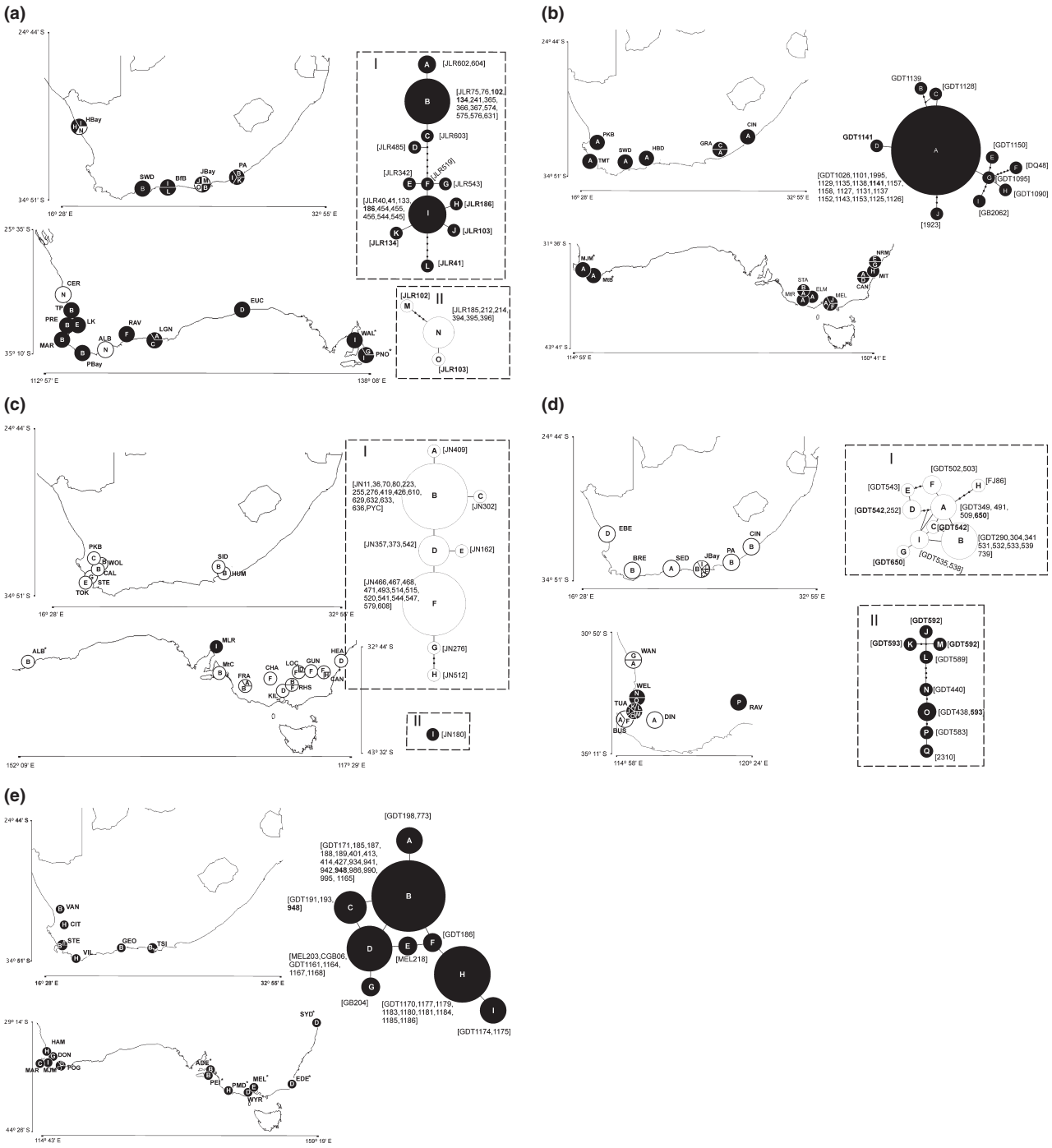

Figure 3 Parsimony networks for the five study species: (a) *Acacia cyclops*; (b) *Acacia mearnsii*; (c) *Acacia pycnantha*; (d) *Acacia saligna*; and (e) *Paraserianthes lophantha*. The networks are based on the external transcribed spacer (ETS) region except for *A. pycnantha* where the rpl32-trnL$^{(UAG)}$ region was analysed. For ETS, allelic copies of heterozygous individuals are indicated in bold accession ID numbers on networks. Pie charts on maps illustrate the proportion of each allele/haplotype at that particular locality. Accessions from other sources for which country of origin is known but no precise locality could be determined are indicated by '§'.

standing genetic diversity (Lachmuth *et al.*, 2010). All acacias in the current study were introduced to South Africa on multiple occasions and, in most cases, substantial genetic diversity was found in the invasive range, with the exception of *A. saligna* and *A. mearnsii*. Even though the latter two have been introduced to South Africa on at least five and eight separate occasions respectively, we found a marked reduction in genetic diversity compared to that found in the native range populations. These findings emphasize how native range genetic structure, sampling effort preceding introduction,

and no prior knowledge of introduction effort, may skew deductions made on introduction history based solely on genetic data (see Fig. 1).

Multiple introduction events may increase the likelihood of cointroducing spatially isolated genetic entities (Dlugosch & Parker, 2008) such as the two major genetic clades identified for *A. cyclops* in Australia (Fig. 3a). Despite substantial geographic separation of some *A. cyclops* genotypes, particularly those sampled in high frequencies (e.g. DNA sequences B and N, Fig. 3a), representatives of all these divergent groups were found growing sympatrically in South Africa. Clearly, the introduction of *A. cyclops* from Australia in 1845 comprised seed containing genetic diversity sampled over a large part of its natural range or, alternatively and in concert, much of the genetic diversity of *A. cyclops* in South Africa could have been introduced indirectly from France in 1895. Such broad-scale geographic sampling prior to single introduction events to South Africa has been documented for other acacias such as *A. mearnsii*, for which seeds were sourced from at least 25 localities throughout its range in Australia prior to introduction (Poynton, 2009). Surprisingly, we only identified two genetic entities (DNA sequence A and C, Fig. 3b) of *A. mearnsii* in South Africa. DNA sequence A was also found in invasive ranges in Australia (sites MJM and MtB,) and was widespread throughout native range regions in south-eastern Australia, often co-occurring with other genetic entities. While it is possible that only a single genetic entity was sampled (see Poynton, 2009), it is also possible that environmental (natural) or human (artificial) selection led to reduced post-introduction genetic diversity. The widespread distribution of genotype A in south-eastern Australia may indicate wider environmental adaptations and tolerance and thus invasibility. *Acacia mearnsii* has been subject to strong artificial selection, particularly for traits associated with growth vigour, in South Africa (K. Nixon, pers. comm.). Many South African introductions of *A. mearnsii* occurred in the eastern parts of the country and more intensive sampling in these areas, specifically commercial plantations, may reveal additional genetic diversity.

Similarly, multiple haplotypes were found in high frequencies in the native ranges of *A. pycnantha* (B and F, Fig. 3c), but only one (haplotype B) was common in the invasive ranges in South Africa. Together with the known introduction history, this may indicate a narrow genetic sampling of *A. pycnantha* prior to introductions.

Species with native ranges in Western Australia (*A. cyclops*, *A. saligna* and *P. lophantha*) all showed high intra-specific diversity in their native ranges. This is consistent with patterns of phylogeographic structure in south-western Australia that have been identified in many species, including acacias (Byrne, 2007). The phylogeographic patterns indicate differing influences of historical climatic conditions with development of aridity in the early to mid Pleistocene leading to highly divergent geographically structured lineages and localized persistence throughout multiple climatic fluctuations since the mid Pleistocene driving high genetic diversity within and between populations (Byrne, 2008). *Acacia saligna* and *A. cyclops* had divergent lineages and patterns of genetic diversity conforming to these expectations (Byrne, 2007). *Paraserianthes lophantha* also showed high diversity but no divergent lineages probably as a consequence of its more restricted natural distribution. In comparison, the south-eastern Australian species, *A. pycnantha* and *A. mearnsii*, showed less diversity and more widespread distribution of common haplotypes (excluding haplotype I of *A. pycnantha*) indicating greater gene flow and less influence of historical climatic cycles in this species from the south-eastern mesic region.

Our results also support the notion that multiple introductions could result in the establishment of genetically diverse populations. While this may also be true for single introduction events (Fig. 1), multiple introductions may enhance the cointroduction of previously allopatric genetic entities with consequences in introduced ranges, such as hybridization (Prentis *et al.*, 2008). The effects of intra-specific hybridization and subsequent hybrid vigour have been demonstrated for numerous invasive species (Ellstrand & Schierenbeck, 2000) and as such represent a potential risk both to precipitating an invasion and to successful management (e.g. biological control). Here, we found evidence for such intra-specific hybridization and genetically novel combinations in at least three species: *A. cyclops*, *A. saligna* and *P. lophantha*. *Acacia cyclops* in South Africa consisted mostly of mixed genotypes representing diverse Australian genetic entities, including at least six heterozygote individuals. In contrast, we did not find a single instance of such admixture in Australia. Whether these unique genetic combinations translate into hybrid vigour and increased invasiveness remains unknown. It is worth noting that our approach may give conservative estimates of the extent of hybridization. Backcrossing and introgression will dilute hybrid signatures inferred from single locus heterozygotes, and so, it will be more difficult to identify such events from an analysis of alleles in a phylogeographic framework. Moreover, homogenization of the ETS region through concerted evolution would furthermore erase evidence of intra-specific hybridization. Here, multiple (up to five) cloned inserts were sequenced in heterozygous individuals, and we never retrieved more than two alleles. We are therefore confident that our results are not confounded by paralogy (also see Brown *et al.*, 2008).

The introduction history, through creating opportunities for hybridization in the invasive range and by potentially selecting particular genotypes, has several important effects on invasive plant management. Most risk assessments ignore information at the sub-specific level. However, introductions of new genetic material can potentially radically increase the invasive range by altering the climatic limits of a species' distribution (e.g. see Thompson *et al.*, 2011). The contribution of sub-specific information in explaining current distributions and levels of invasiveness is a key research topic for Australian *Acacia* species (Wilson *et al.*, 2011). Moreover, if interactions between plants and their herbivores/pathogens co-evolved, then sub-specific information in an invasive plant would be essential for successful biological control (Goolsby *et al.*, 2006a,b).

Geographic and taxonomic placement of invasive populations can assist in identifying more host-specific and/or damaging control agents at the population or even genotype level. Examples of such intra-specificity include the mite biological control agents, *Aceria chondrillae* on skeleton weed (*Chondrilla juncea*) (Cullen *et al.*, 1982; Cullen & Moore, 1983) and *Floracarus perrepae* on the fern *Lygodium microphyllum* (Goolsby *et al.*, 2006a); and the rust fungus, *Phragmidium violaceum* on blackberry, *Rubus fruticosus* agg. (Evans *et al.*, 2005). Similar patterns of specificity have also been documented for the African acacia, *Acacia nilotica* (Palmer & Witt, 2006). Classical biological control is a fundamental component of the long-term integrated control of widespread invasive wattles in South Africa (Moran *et al.*, 2005; van Wilgen *et al.*, 2011). Over 15 control agents have been released against invasive wattle species in South Africa (Impson *et al.*, 2009; see Wilson *et al.*, 2011 for a complete list), largely in the absence of detailed information on the introduction history and phylogeography of the target plant in its native and invasive ranges. For example, the performance of *Dasineura dielsi* Rübsaamen, a gall-forming midge associated with all genetic lineages of *A. cyclops* in Australia (J.J. Le Roux, unpubl. data), is variable in South Africa, with some *A. cyclops* stands being heavily galled while others appear minimally affected (J.H. Hoffmann, pers. comm.). Notably, the original collection localities of *D. dielsi* in Western Australia [Wallaroo/Moonta Bay area (site WA) and in the proximity of Cheyne beach (site AL) (Adair, 2005)] include both major genetic lineages of *A. cyclops* identified in Australia and South Africa. It is possible that the admixture between genetically divergent entities found here could influence host-specificity and/or recognition by *D. dielsi*.

It is clear that introduction history will not only significantly alter immediate but also future adaptive responses of invasive populations. Evolutionary processes in these populations frequently operate following genetic bottlenecks and strong drift and, coupled with stochasticity and post-introduction selection, can radically alter evolutionary outcomes (Keller & Taylor, 2008). For example, introductions from genetically distinct native sources, such as for *A. cyclops*, have led to genotypic novelty, and this may translate into enhanced phenotypic variation for selection to act upon. Multiple introductions may also increase the likelihood of introducing numerous pre- and/or maladapted genotypes, so that selection will swiftly favour only those pre-adapted to the new environment. On the other hand, founding populations may experience genetic bottlenecks; coupled with drift, this could lead to genetically depauperate populations with limited evolutionary potential. These stochastic processes may further blur retrospective inferences made on introduction histories of invasive species from molecular data.

CONCLUSIONS

A full understanding of the behaviour of introduced species requires accurate information on introduction history, including knowledge of the native provenances, geographical pathways and contemporary spread. The lack of detailed historical records often means that indirect approaches must be applied to infer introduction histories and their underlying processes (Le Roux & Wieczorek, 2009; Estoup & Guillemaud, 2010). Our results show that extreme caution must be taken when relying solely on indirect inferences (molecular genetic data) of these processes. This is particularly true for forestry species, such as Australian acacias, that are often characterized by complex introduction histories because of human-mediated selection efforts. For example, single introductions encompassing a mosaic of genetic diversity, sourced from widespread geographical areas in the native range, may, without historical records, be interpreted as multiple introductions. Therefore, thorough introduction records coupled with a thorough understanding of native and invasive range genetic structure are required to reliably reconstruct invasion pathways, especially for commercially introduced species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Locality information for Australian *Acacia* species and *Paraserianthes lophantha*.

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